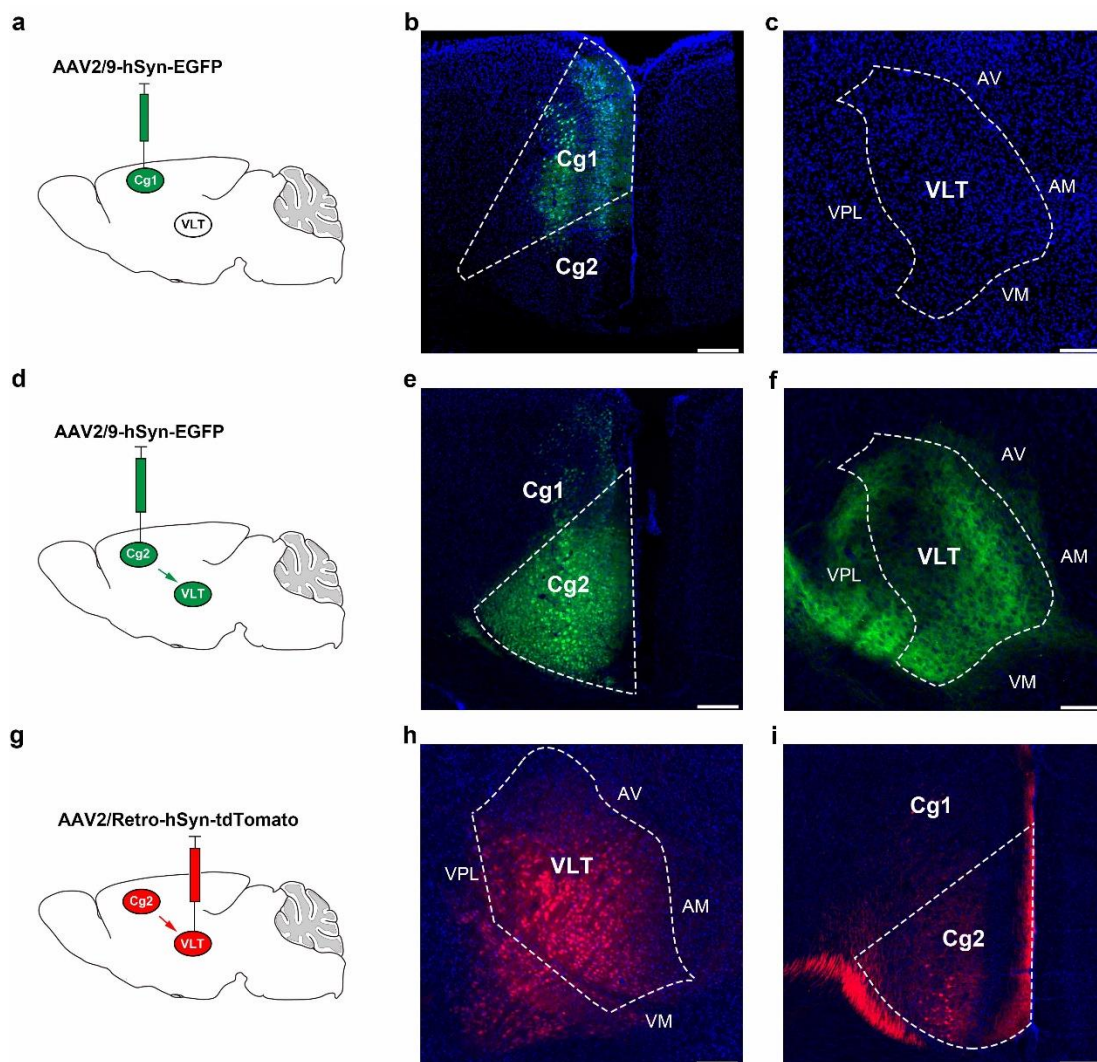


Berberine alleviates chronic pain-induced anxiety-like behaviors by inhibiting the activation of VLT-projecting cACC (Cg2) neurons

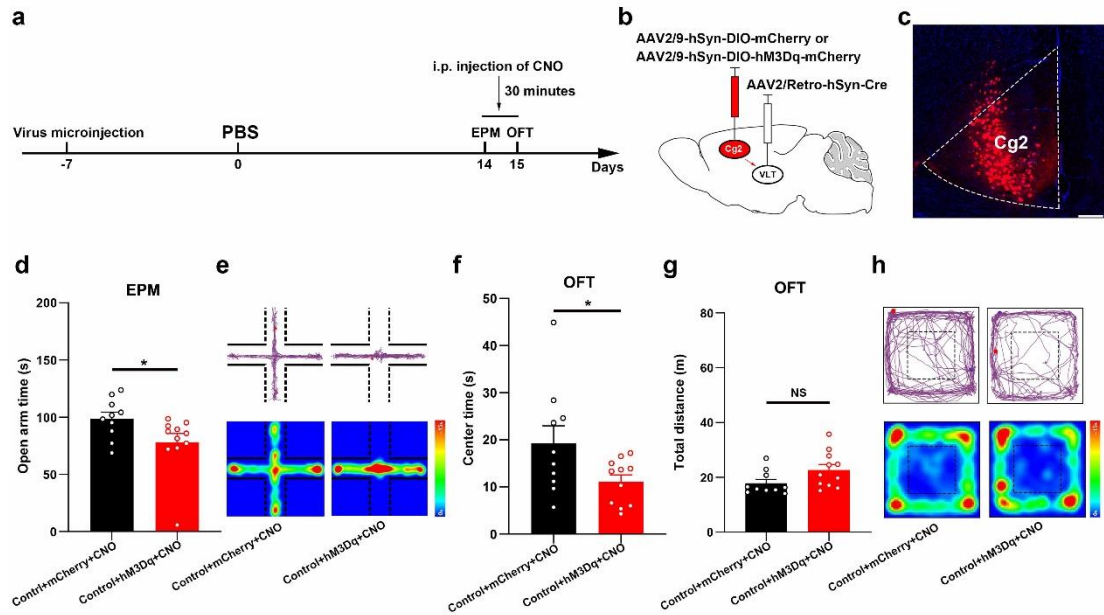
Supplementary Information



Supplementary Figure 1. Neurons in cACC (Cg2), but not cACC (Cg1), project to the VLT.

a Schematic for specific infection of Cg1 neurons with EGFP. **b** Representative images showed that cACC (Cg1) neurons were labeled with green fluorescence, but no projection fibers from cACC (Cg1) neurons were observed in the VLT (**c**). **d** Schematic for specific infection of cACC (Cg2) neurons with EGFP. **e** Representative images showed that cACC (Cg2) neurons were labeled with green fluorescence and the projection fibers from cACC (Cg2) neurons were observed in the VLT (**f**). **g** Schematic for retrograde tracking of projection neurons in cACC from the VLT with tdTomato. **h** Representative images showed

that VLT were labeled with red fluorescence, and that cACC (Cg2) neurons, not cACC (Cg1) neurons, were labeled by retrograde tracing from the VLT (i). Scale bar, 200 μ m. cACC caudal anterior cingulate cortex, Cg2 cingulate area 2, Cg1 cingulate area 1, VLT ventral lateral thalamus.



Supplementary Figure 2. Activation of VLT-projecting cACC (Cg2) neurons induced anxiety-like behaviors in normal mice.

a Schematic of the experimental design. **b, c** Schematic for specific infection of VLT-projecting cACC (Cg2) neurons with mCherry or hM3Dq-mCherry. Scale bar, 200 μ m. **d** Statistical results of time spent in the open arms in the EPM test. **e** Representative images of movement trajectory (top) and activity time (bottom) in the EPM test. **f, g** Statistical results of time spent in the central area and total distance traveled in the OFT. **h** Representative images of movement trajectory (top) and activity time (bottom) in the OFT. Data are the mean \pm SEM; * P <0.05, ** P <0.01, NS=not significant. VLT ventral lateral thalamus, cACC caudal anterior cingulate cortex, Cg2 cingulate area 2, CNO Clozapine N-oxide, EPM elevated plus maze, OFT open field test.